Antibiogram of *Listeria* and *Salmonella* Species Isolated from Tilapia Fish (*Oreochromis nitolicus)* and Snail (*Archachatina marginata*) Sold in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author NNO designed the study, while author VD performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript managed the analyses of the study and literature searches under the strict supervision of authors NNO and DNO. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Land snails are ubiquitous creatures found on farms, forests and domesticated in homes. Bacteriological studies indicate that various potentially pathogenic bacteria inhabit different organs and tissues including the haemolymph of the African giant snail, the presence of pathogenic bacteria in the snails are a health threat to consumers and may cause food borne diseases/ infection. Pollution of aquatic environments with organic waste of animal and human origin may lead to transfer of pathogens to the fishes, thereby making them carriers of the pathogens. In this study antibiogram studies were carried out on the isolates from snail and tilapia fish to determine antibiogram of *Listeria monocytogenes* and *Salmonella* species isolated from tilapia fishes and

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snails sold in Port Harcourt. One hundred and thirty two (132) samples were obtained from three different markets namely; Creek road, Mile one and Rumuokoro markets over a period of six months. Standard analytical protocols were employed to determine the bacteriological characteristics of the various parts such as intestine, flesh/meat, gills and fluid(snail). Antibiogram of *Listeria* and *Salmonella* species were determined using standard methods as recommended by CLSI. Statistical analyses were carried out using ANOVA and All pairs tukey-kramer. Results of Total heterotrophic bacterial count shows that snail samples had the highest number of bacterial count compared to frozen tilapia fishes. Mean *Listeria* spp. count for frozen tilapia fish ranged from 2.7 ±0.68 x10^4cfu/g to 2.9 ±0.23 x10^4cfu/g (flesh), 3.3 ±0.15 x10^4 to 3.7 ±0.35 x10^4cfu/g (gill), 3.8 ±0.44 x10^4 to 4.3 ±0.57 x10^4 cfu/g (Intestine), across the three markets, Mean *Listeria* spp. count for snail sample ranged from 0.7 ±0.29 x10^4 to 1.1 ±0.18 x10^4cfu/ml (Snail fluid) 1.1 ±0.18 x10^4 to 1.2 ±0.16 x10^4cfu/g (meat), 1.6 ±0.44 x10^4 to 1.9 ±0.57 x10^4cfu/g (Intestine) Creek road market. Mean total *Salmonella* count for frozen tilapia fish ranged from 1.0 ±0 x10^3cfu/g to 1.3 ±0.58 x10^3 (flesh), 1.0 ± 0 x10^3 to 1.6 ± 0.58 x10^3cfu/g (gills), 1.2 ±0.5 x10^3 cfu/g to 2 .0 ±1.41 x10^3 cfu/g (Intestine), across the three markets. Mean total *Salmonella* count for snail ranged from 1.0 ±0.58 x10^3 to 1.3 ±0 x10^3cfu/ml (fluid), 1.5 ±1.0 x10^3 to 1.7 ±0.96 x10^3cfu/g (meat), 1.7 ±0.96 x10^3cfu/g to 3.0 ±1.58 x10^3cfu/g (Intestine), across the three markets. Results of antibiogram revealed that all the *Listeria* species were 100% susceptible to Levofloxacin, Ofloxacin, Gentamycin, Azithromycin, Erythromycin and Ceftriaxone-sulbactam but 100% resistance to Augmentin, Ciprofloxacin and Cefuxime while *Salmonella* species were 100% susceptible to Ofloxacin and Ciprofloxacin and resistance to Cetazidime and Gentamicin, thus, these drugs should be considered the drug of choice for infections caused by these bacteria,

1. INTRODUCTION

Land snails are ubiquitous creatures found on farms, forests and in homes. In some communities with poor sewage disposal systems, snails are often found at the sewage disposal sites. The diverse environments in which these snails are found make them susceptible host to a vast range of microorganisms [1]. Thus, they could harbour parasites, bacteria, viruses as well as fungi. Bacteriological studies indicate that various potentially pathogenic bacteria inhabit different organs and tissues including the haemolymph of the African giant snail, [2]. Their presence in the snails is a health threat to consumers [3]. The African giant land snails are mostly found in the forest, farms and gardens where they have unlimited vegetation to feed on. The close contact of snails with soil and organic debris make them susceptible to microbial contamination. Snails are regarded to have high populations of indigenous bacteria also suspected to contain certain poisonous substances which they ingest from their environment [4]. The meat of snails can easily be contaminated by microbial pathogens thereby serving as a vehicle for transmission of infectious agents to consumers.

Tilapia fish is ranked as the second most widely farmed fish in the world [5]. They are farmed in at least 85 countries, with most production coming from Asia and Latin America [6]. The majority (approximately 66.7%) of tilapia production in China is sold alive in domestic market and the remaining are frozen for exportation or used for further processing [6]. The microbiological quality of fishes found in habitats that are polluted by industrial discharge, domestic wastes, anthropogenic activities and others disposal may either kill these fishes or render them inedible which if not taken note of might cause a great deal of harm to man and his environment [5]. Pollution of aquatic environments with organic waste of animal and human origin may lead to transfer of pathogens to the fishes thereby making them carriers of the pathogens. Pathogens that have been found to be associated with fishes that can cause disease in humans include; *Vibrio* spp, *Listeria monocytogenes*, *Yersinia* spp., pathogenic *Salmonella* serovars, and *Clostridium botulinum* [7].

Despite the microbial quality of fishes and snails, they are known as popular protein sources consumed by people worldwide, Apart from being a protein source, fishes are sources of vitamins and minerals [8], while snails are a good source of iron, calcium, and phosphorus, and is said to contain almost all the amino acids needed by humans [4]. But increase in microbial

population especially *Listeria* and *Salmonella* species in food due to improper storage, handling and exposure to contaminants can raise public health concerns for human consumption [9]

Contamination of fish and snails for sale often occur as a result of the way they are handled and exposed in the open markets under poor hygienic conditions. This condition can lead to the increase in inherent microbial load in these food products which upon consumption can lead to food borne diseases, some of which can be fatal. Food borne diseases are prevalent in developing countries and some of the pathogens such as *Salmonella* spp., and *Listeria monocytogenes* that causes these diseases are found in fish and snails. *Salmonella* infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease [9]. Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteraemia and enteric fever [10] *Salmonella* is a rod- shaped, gram-negative facultative anaerobe that belongs to the family Enterobacteriaceae [11]. According to Daminabo et al. [12], genus *Listeria* is a gram-positive, non-spore forming, rod-shaped bacteria of 0.4-0.5x0.5- 2 μm in size with rounded ends and can also be coccoid at times, occurring singly or in short chains and not encapsulated. *Listeria monocytogenes* is reported to be pathogenic in humans and animals and is the causative agent of listeriosis. It is also an agent of several food-borne disease outbreaks and causes serious infection in the elderly, neonates, pregnant women and immune-compromised persons.

An antibiogram is however a chart that shows the susceptibility test result of microorganisms against the tested antibiotics. Due to the increasing rate of resistance of pathogenic organisms to commonly used antibiotics, the need to test microorganisms against these commonly used antibiotics would be of immense help to the physicians in prescribing specific drugs and thereby reduce the use of broad spectrum drugs. The clinical and laboratory standard institute provided guidelines for antimicrobial susceptibility testing and have recommended the development of antibiogram annually. Hence, this study was aimed at providing specific information as regards the antibiogram of *Salmonella and Listeria* species associated with snails and tilapia fishes sold in Port Harcourt markets.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The study was carried out within Port Harcourt city. Port Harcourt is located in the Niger Delta region; Southern Nigeria. The city is situated between latitudes $3^0$37' and $3^0$56' N. and longitude $11^0$10' and $11^0$45' E, approximately 50km from the Atlantic coast. One of Major occupations of the residents/inhabitants of Port Harcourt is fishing around the marine communities in spite of the various exploitation of oil by multinational companies. Image 1 shows the map of Port Harcourt indicating the sampled markets.

2.2 Collection of Samples

Total number of one hundred and thirty two (132) samples of frozen tilapia fish (36), salt water tilapia fish (24) and Land snail (72) samples were obtained from the three different markets namely; Creek road, Mile one and Rumuokoro markets over a period of six months. Sterile containers were used to store the fishes after purchase and then transported to the Laboratory for analyses within 2 hours collection in a thermos box containing ice pack. The samples were collected using sterile bags properly labeled according to standard microbiological procedures as adopted by Omorodion and Odu [11] and Daminabo et al. [12] The fish and snail samples were identified via its feature properties by Dr. Dokuboba Amachree, of the Department of Fishery and Aquatic Environment, Faculty of Agriculture, Rivers State University, Port Harcourt, Nigeria.

2.3 Bacteriological Analyses of Fish and Snail Samples

2.3.1 Preparation of the samples in the laboratory

The fish samples were prepared for bacteriological analysis as described by Danba et al. [13] The fish samples were rinsed with water to remove surface dirt and then the body surfaces were swabbed with ethanol to remove external microorganisms. The skin, gills, and intestine were dissected with the aid of sterile knife and forceps, and separately macerated
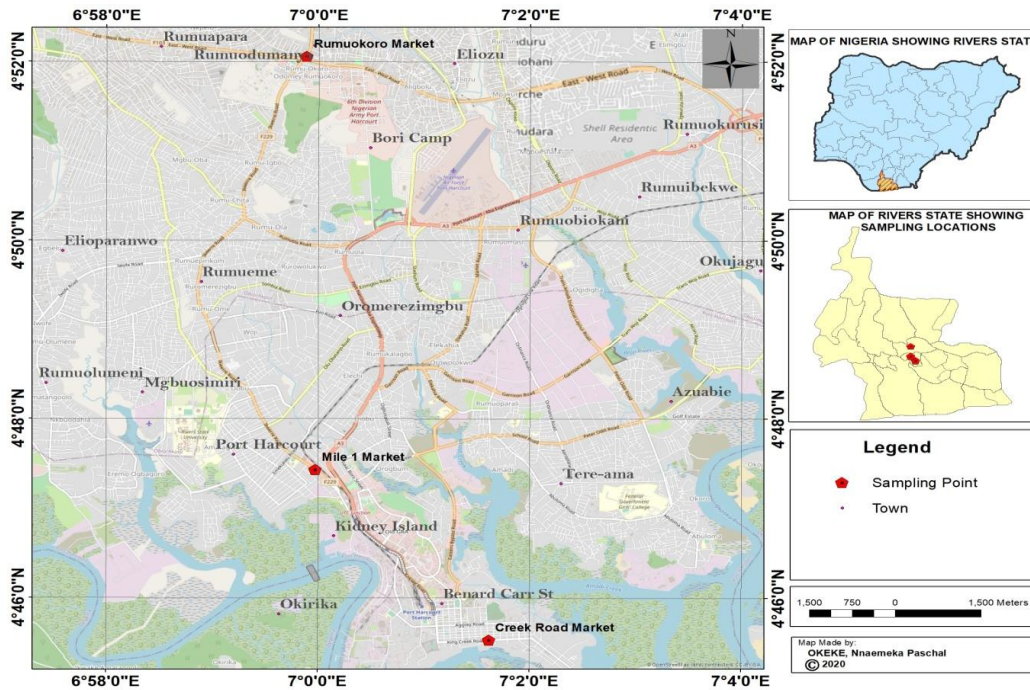
Fig. 1. Map showing Study Area of the various Sampling Markets

aseptically. While the snail samples were prepared for bacteriological analysis as described by Nyoagbe et al. [4] and Bhandare et al. [14]. The snail samples were scrubbed and rinsed with water to remove surface dirt and then washed with sterile distilled water and scrubbed with ethanol to remove external microorganisms. The meats were then aseptically extracted from the shell and homogenized while the fluids were carefully collected in a sterile universal bottle.

2.3.2 Serial dilution

One gram each of the respective samples was separately added to 9 ml of 0.1% peptone water diluents. After thorough shaking, further tenfold (v/v) serial dilutions were made by transferring 1 ml of the original solution to freshly prepared peptone water diluents to a range of 10^{-6} dilutions [13].

2.3.3 Inoculation and Incubation

Aliquots (0.1 ml) of various dilutions were inoculated to surface dried Plates Count Agar (PCA) in triplicates for enumeration of total heterotrophic bacterial population. *Salmonella-Shigella* agar (SSA) for *Salmonella* and *Shigella* populations, and *Listeria* selective agar base supplemented with *Listeria* Selective Supplement

II (FD063 or FD063I) for *Listeria* population in triplicates and spread evenly with flamed bent glass spreader. The plates were incubated at 37^0 C for 24 hours.

2.3.4 Enumeration and Isolation of Pure Culture

2.3.4.1 Total heterotrophic bacteria

Total Heterotrophic Bacteria was enumerated as described by Prescott et al. [15]. Bacterial Colonies that appeared on the respective culture plate containing the various culture medium used were counted and the mean expressed as cfu/g for the respective samples [16]. The colony forming unit per gram of the sample was calculated using the formula below;

$$cfu/g = \frac{\text{number of colonies}}{\text{Dilution x volume plated}}$$

The discrete colonies were sub cultured on fresh Nutrient agar plate in order to isolate pure cultures.

2.3.4.2 Isolation and resuscitation of salmonella species

For isolation and resuscitation of *Salmonella* species, Ten gram (10 g) of the respective snail

samples were agitated in 90 ml sterile peptone broth incubated at 37ºC for 48h., for enrichment, then 10 ml of the incubated peptone containing the sample were transferred into Selenite F broth at 37ºC for 24 hr., for further enrichment, 0.1 ml aliquot was inoculated on *Salmonella-Shigella* Agar (SSA), incubated at 37ºC for 24 hours.

2.3.4.3 Isolation and resuscitation of *Listeria* species

For *Listeria* species resuscitation and isolation, ten grams (10 g) of respective samples was transferred into 90 ml of Half Fraser broth and incubated at 37 ºC for 24 h. Ten mililiter of Half Fraser were transferred into full Fraser broth and incubated at 37 ºC for 24hour, after which 0.1 ml of Full Fraser was inoculated on PALCAM media and incubated at 37 ºC for 24hour.

2.3.4.4 Maintenance of pure culture

Discrete bacterial colonies that grew on the respective media were sub cultured using streak plate method onto freshly prepared nutrient agar and incubated at 37°C for 24 hours in order to obtain pure culture. The pure bacteria cultures were then maintained according to the method adopted by Amadi et al. [17] using ten percent (v/v) glycerol suspension at -4°C.

2.3.4.5 Identification of the *Listeria* and *Salmonella* species

Pure bacterial cultures from the SSA plate and *Listeria* selective agar base supplemented with *Listeria* Selective Supplement II (FD063 or FD063I) after resuscitation was obtained by streaking on freshly prepared nutrient plate agar and incubated for 24 hours. The obtained pure culture of the respective organisms were further characterized and identified based on their morphological and biochemical characteristics [18-21] as well as genomics features*.*

2.4 Antibiogram of the *Listeria* and *Salmonella* Species

The antibiotic susceptibility patterns of the isolates to common antibiotics were evaluated using the Kirby Bauer disc diffusion technique as adopted by Adebayo-Tayo et al. [22]. Zero point five (0.5) MacFarland's 10^8/ml was employed in the inoculum suspensions preparation according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) and Clinical and Laboratory Standards Institute (CLSI) [23]. Mueller-Hinton agar (Difco

Laboratories), Michigan, USA) which medium is the recommended medium for sensitivity analysis by National Committee for Clinical Laboratory Standards (NCCLS). It is an ideal medium for routine antimicrobial susceptibility tests since it shows good batch-to-batch uniformity [18]. Peptone water was prepared; five discrete colonies of the respective identified isolates were inoculated into 5 ml of the 1% peptone broth and incubated at 35°C for 4 – 6 hours after which the inoculum for primary sensitivity testing was prepared from the broth that has been incubated for 4 – 6 hours. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standard. Each of the isolates was uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by spread plate method using sterile swap stick. The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates [23]. The appropriate antibiotic discs were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37ºC for 24h. Interpretation of results was done using the zones of inhibition sizes [23,24]

3. RESULTS AND DISCUSSION

In this study different parts of snail, and frozen tilapia fish from three major markets within Port Harcourt were examined for the presence of bacteria. Result of Total heterotrophic bacterial count shows that snail had the highest number of bacterial count compared to frozen fish (Fig. 2). The highest number of bacteria count was obtained from snail intestine while the least count was from snail fluid across the three markets sampled.

The results obtained in this study are in agreement with the observation of Adebayo et al. [22] who obtained similar bacterial count in their research on microbial quality of different frozen fish sold in Uyo metropolis except that the values obtained in this study were significantly high. These could be attributed to certain factors like temperature which favours some organisms and also handling by vendors and buyers with contaminated hands, unhygienic life style of sellers and poor storage conditions in markets.

Mean *Listeria* spp. count for frozen tilapia fish ranged from 2.7 ±0.68 x10^4cfu/g to 2.9 ±0.23 x10^4cfu/g (flesh), 3.3 ±0.15 x10^4 to 3.7±0.35 x10^4 cfu/g (gills), 3.8 ±0.44 x10^4 to 4.3 ±0.57 x10^4cfu/g

(Intestine), across the three markets, Mean *Listeria* spp. count for snail sample ranged from $0.6 \pm 0.34 \times 10^4$ to $1.1 \pm 0.18 \times 10^4$ cfu/ml (Snail fluid) $0.8 \pm 0.44 \times 10^4$ to $1.2 \pm 0.16 \times 10^4$ cfu/g (Meat) $1.6 \pm 0.44 \times 10^4$ to $1.9 \pm 0.57 \times 10^4$ cfu/g (Intestine). Creek road market samples had more *Listeria* count than Mile 1 and Rumuokoro markets but there were no significant differences except for the count obtained from the gills and snail fluid that showed significant difference at $P < 0.05$. The high count recorded in frozen fish could be attributed to the fact that most *Listeria* spp. are psychrophiles which indicates that they can survive at freezing temperature ranging from $-5°C$ to $-28°C$ especially *Listeria monocytogenes.* Handling and exposure to contaminant by vendors could also contribute to the high *Listeria* load (Fig 3).
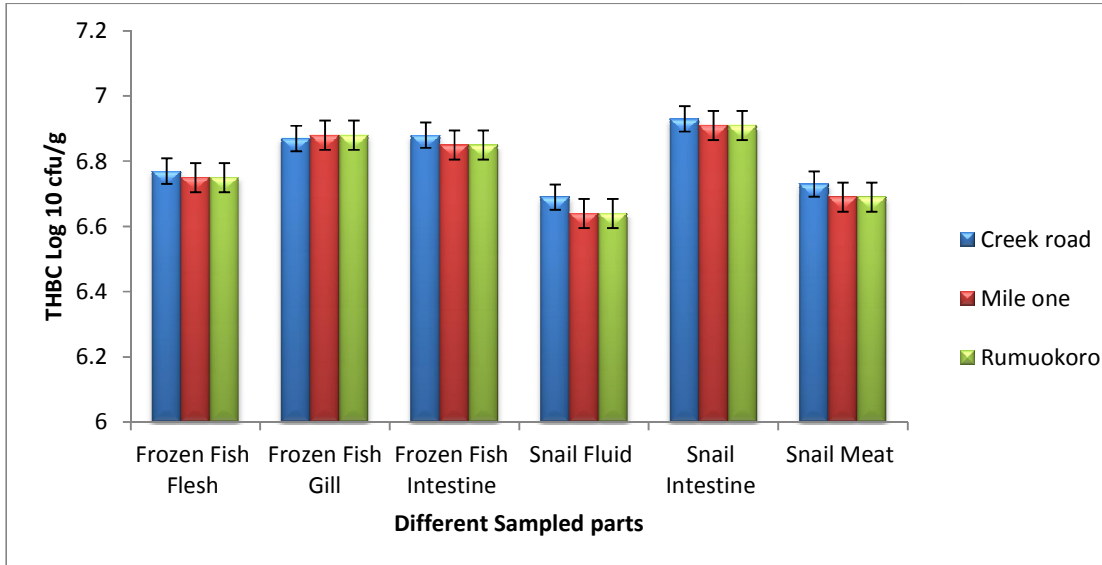

Fig. 2. Total heterotrophic bacteria Count of the various sample parts from the three markets
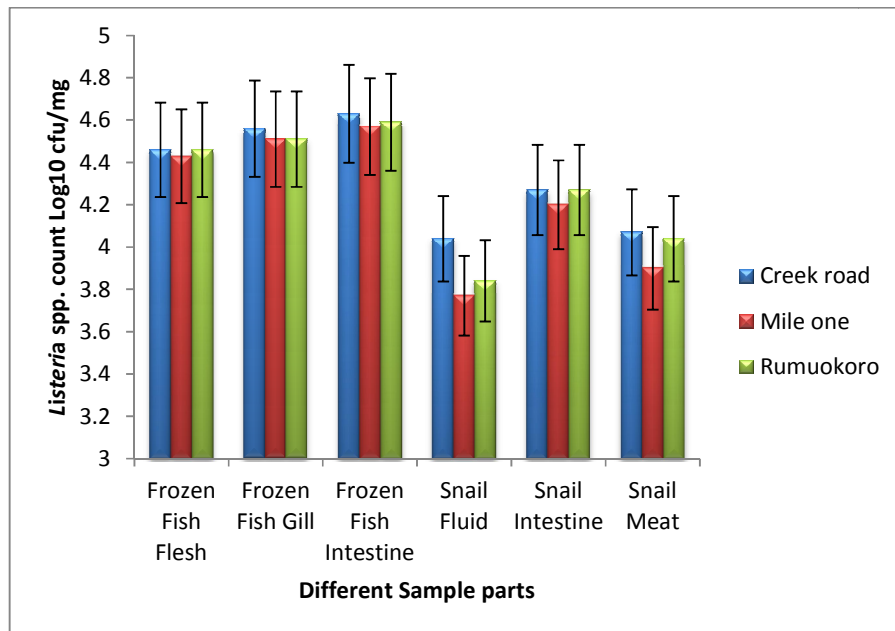

Fig. 3. *Listeria* spp. Count of the various sample parts from the three markets

Mean total *Salmonella* count for frozen tilapia fish ranged from 1.0 x±0 x10^3 cfu/g to 1.3 x±0.58 x10^3 (flesh), 1.0 x10^3±0 to 1.6 ±0.58 x10^3 cfu/g (gills), 1.2 x ±0.5 x10^3 cfu/g to 2.0±1.41 x10^3 cfu/g (Intestine), across the three markets. Mean total *Salmonella* count for snail ranged from 0.4 ±0.58 x10^3 to 1.3 ±0 x10^3 cfu/ml (fluid), 1.5 ±1.0 x10^3 to 1.7 ±0.96 x10^3 cfu/g (meat), 1.5 ±1.15 x10^3 cfu/g to 3.6 ±1.58 x10^3 cfu/g (Intestine), across the three markets. *Salmonella* count of the various samples parts from the three sampled markets shows that at $P<0.05$ there were significant differences between the count obtained from the frozen and snail parts and also within the various samples parts across the three sampled market there were no significant difference at $P <0.05$ (Fig. 4).

These results revealed that total heterotrophic bacteria, *Salmonella* and *Listeria* species counts followed similar trend of having the highest in the intestine and lowest in the flesh for the fishes and fluid for the snail samples. This may be because of the high nutritional value stored in the intestines and the low composition in the flesh and fluid [24].

The study of antibiotic resistance amongst bacterial pathogens isolated from snail and fish is essential, as it might indicate the extent of alteration of water and soil ecosystems by anthropogenic activities. Actually, soil and water bacteria could be autochthonous to aquatic and land environments, or exogenous, transiently and occasionally present in the soil and water as a result of shedding from animal, vegetal, or soil surfaces. The recalcitrance of bacterial strains to antimicrobials could be explained by the possibility of the heavy use of these compounds in aquaculture and agriculture, several of which are non-biodegradable, thus increasing antibiotic selective pressure in soil and water, facilitating the transfer of antibiotic-resistant determinants between aquatic and terrestrial bacteria [25].

Antibiotics susceptibility testing carried out on *Salmonella* spp. isolated in this study showed one hundred per cent 105(100%) resistance to at least one antibiotic across the three locations studied. All tested *Salmonella* spp showed a 105(100%) resistance to Cetazidime (CAZ) across the markets with resistance of isolates from Rumuokoro contributing 23(100%); mile one 35(100%) and Creek road 47(100%). These records differs from report by Bulbu et al. [26] who reported 16(100%) sensitivity of all *Salmonella* isolated in their study without any being resistant to Cetazidime (CAZ). Contrarily, Islam et al. [27,] reported a 5(31.25) resistance to CAZ in their study on antibiotics profile of bacteria isolates from fish samples.

Similar records taken for Cetazidime, a 105(100%) level of resistance to Gentamycin (GEN) was revealed. Isolates from Rumuokoro market showed 23(100%) resistance while Creek road and Mile one contributed 35(100%) and 47(100%) respectively to the overall 105(100%) resistance to Gentamycin recorded. This result is in disagreement with previous studies that
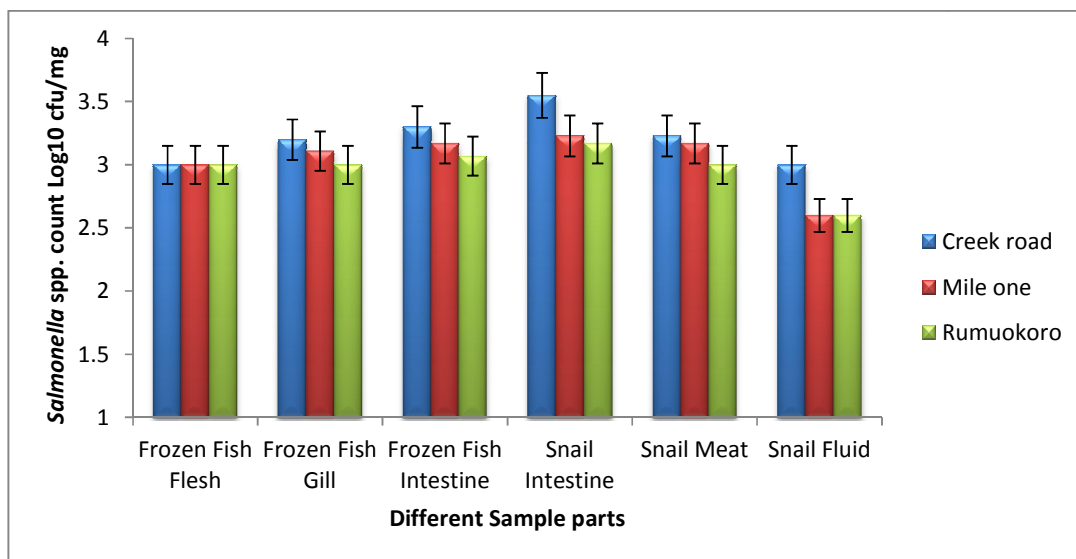

Fig. 4. *Salmonella* spp. Count of the various sample parts from the three markets

revealed a 14(100%) susceptibility to *Salmonella* spp. to the antibiotic as reported by Tamba et al*.,* [28] in Zaria, Nigeria. The report of Islam et al*.* [27] is not in agreement with the findings in this study, Islam et al*.* [27] reported 16(100%) susceptibility of *Salmonella* spp. to Gentamycin without any resistance recorded. Notwithstanding, our study corroborates with report by Jambo et al. [29] who recorded a low 6.9% susceptibility to Gentamycin with a 93.1% resistance to the antibiotics. Level of resistance against Gentamycin was very much in agreement to the findings of Samantha et al*.* [30] who found 100% of their *Salmonella* isolates resistant Gentamycin in india.

Isolates from Rumuokoro market showed a 23(100%) resistance to Cloxacillin (CXC) but varying resistance pattern of 38(80.8%) and 18(51.4%) for *Salmonella* spp. isolated from samples sourced from Creek road and Mile one markets respectively. However, the overall resistance to CXC was 79(75.2%) with an intermediate pattern of 26(24.8%) suggesting a sharp variation from the 43% resistance of *Salmonella* to Cloxacillin reported by Claudious et al*.* [31]. None of the isolates from all the source studied were susceptible to Cloxacillin.

Antibiotics susceptibility pattern of *Salmonella* isolates to other antibiotics showed varying pattern of susceptibility. An overall 19(18.1) resistance to Augmentin with a 53(50.5%) susceptibility was recorded with Rumuokoro contributing 5(21.7%), Creek road 10(21.3%) and mile one 4(11.4%) of the resistance reported Ciprofloxacin (CPR) and Ofloxacin (OFL) were 100% potent against all *Salmonella* species isolated from the three locations. This is not in agreement with the 15(46.87) low susceptibility and 5 (15.62%) resistance to Ciprofloxacin (CPR) reported by Irfan et al*.* [32]. However the overall 100% susceptibility of *Salmonella* spp. recorded corroborates report by Selvaraj et al*.,* [33] who reported 100% susceptibility of *Salmonella* spp. from food samples to Ofloxacin (OFL).

Data revealed levels of antibiotic resistance form, Erythromycin (ERY) showed total resistance of 14(13.3%) with the bulk resistance percentage recorded for samples sourced from Creek road 10(21%)]. However, Mile one contributed 4(11.4%) while data from Rumuokoro only had intermediate 14(60.9%) and sensitive 9(39.1) levels of anti-erythromycin susceptibility pattern.

Previous studies by Claudious et al. [31] revealed a (65%) resistance to Erythromycin (ERY) contradicting our record in this study. There were no significant difference for levels of resistance, intermediate and sensitivity to Nitrofurantoin (NIT) by *Salmonella* spp. isolates in the three markets studied at p<0.005. however the overall resistance level of isolates to NIT was 11(10.5%) while 45(42.9%) and 49(46.6%) where recorded for intermediate and sensitive levels respectively. *Salmonella* spp isolated in this study were more resistant to the Cephalosporins and Aminoglycosides in terms of classes. Table 1.

Listeria spp. isolated in this study were also tested for their sensitivity to various antibiotics. Among the isolates the high in vitro sensitivity was shown by 409 (100%) of isolates for Gentamicin, Ceftriaxone /Sulbactam, Levofloxa cin, Ofloxacin, Azithromycin and Erythromycin. The isolates were however found to be 409(100%) resistant to Ciprofloxacin, Amoxicillin/ Clavulanic, Imipenem/Cilastatin, Cefuxime and Cefotaxime. The 100% susceptibility level recorded for Gentamicin and Ceftriaxone/Sulbactam corroborates Bulbul et al*.* [26], who also reported 100% susceptibility of *Listeria* spp. to Gentamycin. The findings of this study however disagree with their 100% sensitive report for Erythromycin. Differences in the antimicrobial susceptibility pattern recorded in this study when compared to some studies done previously could be as a result of exposure of these organisms to antibiotic used in aquaculture/agriculture which may cause changes in their genome and also, possibly due to chemical and harsh environmental conditions where they live as their habitat, thus acquiring plasmids which enable these organisms transform to strains able to resist antibiotics they are normally susceptible to.

The underlying mechanism of β-lactam antibiotic resistance is through the production of β-lactamases. These enzymes function by hydrolysing the β-lactam ring by breaking the amide linkage, thereby disabling their capability of inhibiting bacterial cell wall synthesis [28]. Previously, it was known that ESBL-producing bacteria thrived commonly in hospital and clinical settings where extensive use of antimicrobial drugs and agents assisted in the development of their antimicrobial resistance [33].

Table 1. Antibiotic Sensitivity pattern of *Salmonella* spp. isolated from the markets

Antibiotics	Rumuokoro (n=23)			Mile One (n=35)			Creek Road (n=47)			Overall sensitivity report across all markets (N=105)		
	R	I	S	R	I	S	R	I	S	R	I	S
CRX	0(0)	7(30.4)	16(69.6)	2(5.7)	5(14.3)	28(80)	5(10.6)	10(21.3)	32(68.1)	7(6.7)	22(21)	76(72.4)
AUG	5(21.7)	12(52.2)	6(26.1)	4(11.4)	11(31.4)	20(57.1)	10(21.3)	10(21.3)	27(57.4)	19(18.1)	33(31.4)	53(50.5)
NIT	7(30)	3(13.4)	13(56.6)	4(11.4)	18(51.4)	13(37.2)	0(0)	24(51.1)	23(48.9)	11(10.5)	45(42.9)	49(46.6)
CPR	0(0)	0(0)	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)
CAZ	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)	0(0)	0(0)
GEN	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)	0(0)	0(0)
CXM	5(21.7)	7(30.5)	11(47.8)	4(11.4)	21(60)	10(28.6)	9(19.1)	14(29.8)	24(51.1)	18(17.1)	42(40)	45(42.9)
OFL	0(0)	0(0)	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)
CTR	5(21.7)	8(34.8)	10(43.5)	4(11.4)	21(60)	10(28.6)	5(10.6)	19(40.5)	23(48.9)	14(13.3)	48(48.7)	43(40.9)
ERY	0(0)	14(60.9)	9(39.1)	4(11.4)	14(40)	17(48.6	10(21.3)	14(29.8)	23(48.9)	14(13.3)	42(40)	49(46.7)
CXC	23(100)	0(0)	0(0)	18(51.4)	17(48.6)	0(0)	38(80.8)	9(19.1)	0(0)	79(75.2)	26(24.8)	0(0)

Key: AUG; Augmentin, NIT; Nitrofurantion, CPR; Ciprofloxacin, CAZ; Cetazidime,; GEN.; Gentamicin, CXM.; Cefixime, OFL.; Ofloxacin, CTR.; Cftriaxone, ERY; Erythromycin, CXC.; Cloxacillin. CRX; Cefuroxi.
Values in parenthesis represents the percentage susceptibility to antibiotics

Table 2. Antibiotics Sensitivity pattern of *Listeria* spp. isolated from the markets

Antibiotics	Rumuokoro (n=23)			Mile One (n=35)			Creek Road (n=47)			Overall sensitivity report across all markets (N=105)		
	R	I	S	R	I	S	R	I	S	R	I	S
Amoxicillin/Clavulanate	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)	0(0)	0(0)
Cefotaxime	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)	0(0)	0(0)
Ceftriazone sulbactam	0(0)	0(0)	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)
Cefexime	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)	0(0)	0(0)
Levofloxacine	0(0)	0(0)	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)
Ofloxacin	0(0)	0(0)	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)
Gentamycin	0(0)	0(0)	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)
Azithromycin	0(0)	0(0)	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)
Erythromycin	0(0)	0(0)	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)

Antibiotics	Rumuokoro (n=23)			Mile One (n=35)			Creek Road (n=47)			Overall sensitivity report across all markets (N=105)		
	R	I	S	R	I	S	R	I	S	R	I	S
Imipenem	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)	0(0)	0(0)
Cefuxime	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)	0(0)	0(0)
Ciprofloxacin	103(100)	0(0)	0(0)	136(100)	0(0)	0(0)	170(100)	0(0)	0(0)	409(100)	0(0)	0(0)

4. CONCLUSION AND RECOMMENDATION

There were significant differences in the bacteriological load between frozen tilapia fish and snail evaluated but no significant differences across the three sampled markets at (P = 0.05). The high total viable counts obtained in this study strongly suggest the urgent need to improve the quality control and assurance systems. The results of this study also constitute an indicator of bacteriological contamination of variety of fishes and snails.

Results of antibiogram revealed that all the *Listeria* species were 100% susceptible to Levofloxacin, Ofloxacin, Gentamycin, Azithromycin, Erythromycin and Ceftriaxone-sulbactam but 100% resistance to some of the most commonly use broad spectrum antibiotics such as Augmentin, Ciprofloxacin and Cefuxime while *Salmonella* species were 100% susceptible to Ofloxacin and Ciprofloxacin and completely resistance to Cetazidime and Gentamycin, thus, these drugs should be considered the drug of choice for infections caused by these bacteria. Due to high resistance of *Listeria* species to common preservative methods, such as the use of salt, smoke or acidic condition in the food, and its ability to survive and grow at refrigeration temperatures (around 5 °C). All sectors of the food chain should be Implement Good Hygienic Practices (GHP). Proper blanching and heating methods should be employed during preparations of fishes and snaisl to avoid cross contamination and food intoxication / poisoning before consumption.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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